REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 24, 2002	Final Report February 1990-30 Sept.9

4. TITLE AND SUBTITLE
Fiber Optic Metal Ion Biosensor

5. FUNDING NUMBERS
N00014-91-J-1572

6. AUTHOR(S)
Richard B. Thompson

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Maryland School of Medicine
108 N. Greene Street
Baltimore, Maryland 21201

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution unlimited

20020614 167

13. ABSTRACT *(Maximum 200 words)*

This report describes the initial conception and reduction to practice of a fluorescence-based fiber optic metal ion biosensor. The sensor transducer is a variant of a biologically derived molecule, human apocarbonic anhydrase II, which binds metal ions such as Cu(II), Zn(II), Cd(II), Co(II), and Ni(II) with high affinity and specificity. Several approaches were developed to transduce the presence or level of the metal ion as a change in fluorescence intensity, wavelength, lifetime, or anisotropy (polarization). Sensors were constructed capable of determining Zn(II) and Cu(II) at part per trillion levels in the presence of Ca(II) and Mg(II) at part per thousand levels.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Fluorescence, Metal Ion, Biosensor, Copper, Zinc, Fiber Optic	12
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclass	Unclass	Unclass	UL

FINAL REPORT

Grant No. N00014-91-J-1572

PRINCIPAL INVESTIGATOR: Richard B. Thompson

INSTITUTION: University of Maryland School of Medicine

GRANT TITLE: Fiber Optic Metal Ion Biosensor

AWARD PERIOD: 01 February 1990 to 30 September 1999

OBJECTIVE: The central objective of this project was to see if a fluorescence-based sensor employing a biological or biomimetic molecule as a transducer could demonstrate high sensitivity, selectivity, as well as rapid and reversible response to an analyte such as Cu(II) or Zn(II). A key goal was to see if the sensor figures of merit could be optimized by modification (mutagenesis)of the transducer molecule; among the figures of merit were the affinity, selectivity, stability, and response speed of the sensor. Results of that work are described in the ONR grant Final Reports of Drs. Carol Fierke and David Christianson.

APPROACH: The basic approach was to take advantage of the known propensity of human apocarbonic anhydrase II to bind certain metals in its active site. The approximate affinities of the wild type enzyme are Cu(II): 0.1 pM, Zn(II): 4 pM, Cd(II): 10 nM, Ni(II): 40 nM, and Co(II): 100 nM; importantly, neither Ca(II) nor Mg(II) appear to bind at all, a key consideration for measurements in sea water or serum, where these cations are present at millimolar levels. Because the binding is reversible, the fractional saturation of the binding site is a simple function of the free metal ion concentration and therefore is easily calculated from it.

Several means were developed to transduce the binding of particular metal ions as changes in fluorescence intensities, spectra, lifetimes, or anisotropies (polarizations). Chronologically the first approach was that of Thompson and Jones, which took advantage of the results of Chen and Kernohan, wherein a fluorescent aryl sulfonamide inhibitor for carbonic anhydrase, dansylamide, was found that bound as a fourth ligand to the active site Zn(II). Thompson and Jones found that dansylamide did not bind in the absence of zinc, and thus the fractional occupancy of the binding site and the associated free zinc concentration are easily determined from changes in the fluorescence intensity, spectrum, and lifetime. The dynamic range of zinc determination could be expanded by varying the wavelength at which the lifetime is measured.

This approach was improved upon by the development of other fluorescent aryl sulfonamides with better fluorescence properties, such as ABD-N, ABD-M, Dapoxyl sulfonamide, and BTCS. We constructed a fluorescent energy transfer-based transducer by attaching a fluorescent label to the apoprotein and using a colored aryl sulfonamide which could serve as an energy transfer acceptor for the label; in the presence of zinc the acceptor would bind and accept the labels's energy, reducing the lifetime and intensity, whereas in the absence of zinc no energy transfer occurs and the label remains unquenched. We showed that the response of such transducers can be optimized by placement of the label using site-directed mutagenesis.

We showed that similar sensitivity could be achieved in a "reagentless" format without the diffusible aryl sulfonamide small molecule, and this sensor responded to metal ions other than zinc. In particular, Cu(II), Ni(II), and Co(II) exhibit weak d-d absorption bands which are too weak (extinction coefficients one hundred to one thousand-fold weaker than typical organic dyes)to be analytically useful, but which serve admirably as energy transfer acceptors. Thus by placing the donor label in a suitable position and choosing its spectral properties to overlap well with the metal absorbance, very large responses were achievable, and free Cu(II) concentrations were detectable down in the sub-picomolar (parts per quadrillion) range. The protein transducer could be incorporated into a lifetime-based optical fiber sensor.

While lifetime-based sensing is well-suited to remote sensing using optical fiber, measurements of fluorescence lifetimes in the nanosecond range require relatively complex and costly instrumentation. Because of this, we demonstrated anisotropy-based determinations of metal ions using a similar approach, but which only requires simple steady state measurements. This approach was demonstrated using both reagentless and reagented configurations. The approach was also demonstrated with two-photon excitation using infrared excitation from a mode-locked titanium sapphire laser.

More recently, these approaches were adapted to measuring free copper in sea water at sub-picomolar levels in real time through a length of optical fiber.

Related work was carried out under ONR grants N00014-94-1-0353, N00014-98-1-0475, N00014-98-1-0685, and N00014-00-1-0921 (all to Richard Thompson), and grants awarded to Carol A. Fierke (N00014-93-1-1245 and N00014-95-1-0573) and David W. Christianson.

ACCOMPLISHMENTS: While the details of the program accomplishments

are described in greater detail in the listed references, a few "firsts" may be noted. While not the first fluorescene-based metal ion biosensor, our sensor remains the most selective and sensitive sensor of any kind for zinc, and probably for copper. We were the first to demonstrate a ratiometric metal ion biosensor, a lifetime-based metal ion sensor, an energy transfer-based metal ion sensor, and the determination of metal ions of any kind by fluorescence anisotropy. Our colleagues Carol Fierke and David Christianson were the first to show that the metal ion affinity, selectivity, and/or binding kinetics could be dramatically improved by subtle changes in the protein structure, arrived at either by design or combinatorially. By comparison, this remains difficult to achieve in organic fluorescent indicators for zinc and copper. Twenty years after the development of Fura-2, the most sensitive fluorescent indicators for zinc (Fura and TPEN derivatives from several laboratories) are still one hundred-fold less sensitive than ours, and the few (Newport Green and FuraZin-1)that are sufficiently selective to be used in the presence of millimolar calcium and magnesium are one million-fold less sensitive than ours. While not the first to demonstrate real time sensing of copper in sea water, we were the first to show that fluorescence anisotropy determinations could exhibit an expanded dynamic range, and the first to image the release of zinc in real time from the brain in response to an electrical stimulus.

CONCLUSIONS On the basis of this work we conclude that fluorescence-based biosensors employing variants of carbonic anhydrase as the transducer molecule are capable of determining free metal ions such as Cu(II) and Zn(II) at picomolar levels and Co(II), Ni(II), and Cd(II) at nanomolar levels in real time, through a length of optical fiber, in media as complex as sea water or cerebrospinal fluid.

SIGNIFICANCE This work shows that biological molecules are not only workable but preferred as sensitive and selective recognition elements in biosensors, and offer the best hope for sensing (e.g., continuous, real-time determination of an analyte) in complex media. While we have transduced the recognition as a change in fluorescence which can be measured through a length of optical fiber, transduction could also be electrochemical, or by other means. It also suggests that biosensors may be useful in a variety of applications currently not served by existing technology, particularly environmental monitoring and clinical sensing.

PATENT INFORMATION:

R.B. Thompson, D. Elbaum, V.L. Feliccia, D.W. Christianson, M.W. Patchan, Z. Ge, B.P. Maliwal, "Determination of metal ions in solution by photoluminescence anisotropy,", U.S. Patent No.

6,284,594 (2001).

R. B. Thompson, M. W. Patchan, Z. Ge, "Enzyme-based fluorescence biosensor for chemical analysis," U. S. Patent No. 6,225,127 B1 (2001).

R. B. Thompson, B. P. Maliwal, V. L. Feliccia, C. A. Fierke, "Photoluminescent sensors of chemical analytes," U. S. Patent No. 6,197,258 B1 (2001).

R. B. Thompson, M. W. Patchan, and Z. Ge, "Enzyme-based fluorescence biosensor for chemical analysis," U. S. Patent No. 5,952,236 (1999).

R.B. Thompson and E.R. Jones, "Selective Metal Ion Detection Using a Photoluminescent Indicator Binding to a Macromolecule-Metal Ion Complex," U.S. Patent No. 5,545,517 (1996).

AWARD INFORMATION: None

PUBLICATIONS AND ABSTRACTS:

R. B. Thompson, W. O. Whetsell, B. P. Maliwal, C. A. Fierke, and C. J. Frederickson, "Fluorescence microscopy of stimulated Zn(II) release from organotypic cultures of mammalian hippocampus using a carbonic anhydrase-based biosensor system," *Journal of Neuroscience Methods* **96** 35-45 (2000).

R. B. Thompson, B. P. Maliwal, and H.-H. Zeng, "Zinc biosensing with multiphoton excitation using carbonic anhydrase and improved fluorophores," *Journal of Biomedical Optics* **5**(1) 17-22 (2000).

C. J. Frederickson, S. W. Suh, D. Silva, C.J. Frederickson, and R. B. Thompson, "Importance of zinc in the central nervous system: the zinc-containing neuron," *J. Nutrition (supplement)* **130** 1471S-1483S (2000).

J.A. Hunt, C.A. Lesburg, D.W. Christianson, R.B. Thompson, and C.A. Fierke, "Active-site engineering of carbonic anhydrase and its application to biosensors," in *The Carbonic Anhydrases: New Horizons* (W. R.. Chegwidden, N. D. Carter, and Y. H. Edwards, eds.), Basel: Birkhauser Verlag, pp. 221 - 240 (2000).

R. B. Thompson, H.-H. Zeng, M. Loetz, and C. A. Fierke, "Issues in enzyme-based metal ion biosensing in complex media," in *Proceedings of the SPIE Conference on In-vitro Diagnostic Instrumentation* Vol. 3913 (G. E. Cohn, ed.) pp. 120-127 (2000).

R. B. Thompson, B. P. Maliwal, and H.-H. Zeng, "Improved fluorophores for zinc biosensing using carbonic anhydrase," in

Proceedings of the SPIE Conference on New Technologies in Clinical Diagnostics and Drug Discovery II, Vol 3603 (G. Cohn and J. C. Owicki, eds.), pp. 14 - 22 (1999).

R. B. Thompson, H.-H. Zeng, M. Loetz, K. McCall, and C. A. Fierke, "Improved response of a fluorescence-based metal ion biosensor using engineered carbonic anhydrase variants," in *Proceedings of the SPIE Conference on Advanced Materials and Optical Systems for Chemical and Biological Detection*, Vol. 3858 (M. Fallahi and B. I. Swanson, eds.) pp. 161-166 (1999).

K. Listiak, S. W. Suh, B. Bell, J. Chen, D. Silva, M. Motamedi, G. Schneider, W. Whetsell, R. B. Thompson, and C. J. Frederickson, "Detection of pathological zinc accumulation in neurons: methods for autopsy, biopsy, and cultured tissue," *Journal of Histochemistry and Cytochemistry* **47**(7) 969-972 (1999).

R.B. Thompson, B. P. Maliwal, and C. A. Fierke, "Selectivity and sensitivity of fluorescence lifetime-based metal ion biosensing using a carbonic anhydrase transducer," *Analytical Biochemistry* **267**, 185-195 (1999).

R. B. Thompson, B. P. Maliwal, and C. A. Fierke, "Fluorescence-based sensing of transition metal ions by a carbonic anhydrase transducer with a tethered fluorophore," in *Proceedings of the SPIE Conference on Advances in Fluorescence Sensing Technology IV*, Vol. 3602 (J.R. Lakowicz, R. B. Thompson, and S. A. Soper, Eds.), pp. 85-92 (1999).

R.B. Thompson, B. P. Maliwal, V. L. Feliccia, C. A. Fierke, and K. McCall, "Determination of picomolar concentrations of metal ions using fluorescence anisotropy: biosensing with a "reagentless" enzyme transducer," *Analytical Chemistry* **70**, 4717-4723 (1998).

R.B. Thompson, B.P. Maliwal, V.L. Feliccia, and C.A. Fierke, "High sensitivity determination of Zn(II) and Cu(II) *in vitro* by fluorescence polarization," in *Proc. SPIE Conference on Systems and Technologies for Clinical Diagnostics and Drug Discovery, Vol 3259* (G. Cohn, Ed.), pp. 40-47 (1998).

R. B. Thompson, B. P. Maliwal, and C.A. Fierke,"Expanded dynamic range of free zinc ion determination by fluorescence anisotropy," *Analytical Chemistry* **70** 1749-1754 (1998).

R.B. Thompson, "Biomedical fiber optic sensors: problems and prospects," in *Optical Fiber Sensor Technology IV: Devices and Applications* (K.T.V. Grattan and D.L. Meggitt, eds.) London:

Kluwer Academic Publishers, pp. 67 - 85 (1998).

R. B. Thompson, B. P. Maliwal, and C. A. Fierke, "Determination of metal ions by fluorescence anisotropy exhibits a broad dynamic range," in *Proc. SPIE Conference on Advances in Optical Biophysics, Vol 3256* (J. R. Lakowicz and J. B. A. Ross, Eds.), pp. 51-59 (1998).

R.B. Thompson, H.-J. Lin, Z. Ge, K. Johnson, and C.A. Fierke, "Fluorescence lifetime-based determination of anions using a site-directed mutant enzyme transducer," in *Proc. SPIE Conference on Advances in Fluorescence Sensing Technology III, Vol. 2980* (R.B. Thompson, ed.), pp. 247-256 (1997).

R.B. Thompson, editor, *Proceedings of the SPIE Conference on Advances in Fluorescence Sensing Technology III, Vol. 2980* Bellingham, WA: Society of Photooptical Instrumentation Engineers, pp. 582 (1997).

R.B. Thompson, M.W. Patchan, Z. Ge, and C.A. Fierke, "Fluorescence-based fiber optic chemical sensors," in *Proceedings of the Fifth Biennial Department of Defense Photonics Conference* (R. Kochanski, L. Coryell, and B.M. Hendrickson, eds.) McLean, VA: Armed Forces Communications and Electronics Association, pp. 67-70 (1996).

R.B. Thompson, Z. Ge, M. W. Patchan, C.A. Fierke, K.A. McCall, D. Elbaum, and D.W. Christianson, "Determination of multiple analytes using a fiber optic biosensor based on fluorescence energy transfer," *Proc. SPIE Conf. Ultrasensitive Clinical Diagnostic Systems, Vol. 2680* (G.A. Cohn, ed.), pp. 47-56 (1996).

D. Elbaum, S.K. Nair, M.W. Patchan, R.B. Thompson, and D.W. Christianson, "Structure-based design of a sulfonamide probe for fluorescence anisotropy detection of zinc with a carbonic anhydrase-based biosensor," *J. Am. Chem. Soc.* 118, 8381-7 (1996).

R.B. Thompson, Z. Ge, M.W. Patchan, and C.A. Fierke, "Performance enhancement of fluorescence energy transfer-based biosensors by site-directed mutagenesis of the transducer," *J. Biomed. Optics* 1(1), 131-137 (1996).

R.B. Thompson, Z. Ge, M.W. Patchan, C.-C. Huang, and C.A. Fierke, "Fiber optic biosensor for Co(II) and Cu(II) based on fluorescence energy transfer with an enzyme transducer," *Biosensors and Bioelectronics* 11 (6/7), 557-564 (1996).

R.B. Thompson and M.W. Patchan, "Lifetime-Based Fluorescence

Energy Transfer Biosensing of Zinc," *Anal. Biochem.* 227, 123-128 (1995).

R.B. Thompson and M.W. Patchan, "Fluorescence Lifetime-Based Biosensing of Zinc: Origin of the Broad Dynamic Range," *J. Fluoresc.* 5, 123-130 (1995).

R.B. Thompson, Z. Ge, M.W. Patchan, and C.A. Fierke, "Energy transfer-based fiber optic metal ion biosensor," *Proc. SPIE Conf. Advances in Fluorescence Sensing Technology (SPIE Vol. 2388)* (J.R. Lakowicz, ed.), pp. 138-147 (1995).

R.B. Thompson, Z. Ge, M.W. Patchan, L.L. Kiefer, and C.A. Fierke, "Site-specific Mutants of Carbonic Anhydrase for Fluorescence Energy Transfer-based Metal Ion Biosensing," in *Proc. SPIE Conf. on Chemical, Biological, and Environmental Fibre Optic Sensors VII (SPIE Vol. 2508)* (A. V. Scheggi. ed.) 136-144 (1995).

R.B. Thompson and D.R. Walt, "Emerging Strategies for Molecular Biosensors," *ONR Research Reviews* 46 (3), 19-29 (1994).

R.B. Thompson, "Red and Near-Infrared Fluorometry," in *Topics in Fluorescence Spectroscopy Vol. 4: Probe Design and Chemical Sensing* (J.R. Lakowicz, ed.) New York, Plenum Press, pp. 151-181 (1994).

R. B. Thompson, "FIber Optic Chemical Sensors," *IEEE Proc. Circ. Dev.* 10 (3), 14-21 (1994).

R.B. Thompson and M.W. Patchan, "Fluorescence Lifetime-Based Sensing of Zinc in Solution," in *Proceedings of the SPIE Conference on Chemical, Biochemical, and Environmental Fiber Sensors V, Vol. 2068* (R.A. Lieberman, ed.) pp. 296-306 (1994).

R.B. Thompson and E.R. Jones, "Enzyme-Based Fiber Optic Zinc Biosensor," *Analytical Chemistry* 65, 730-4 (1993).

R.B. Thompson and J.R. Lakowicz, "Fiber Optic pH Sensor Based on Phase Fluorescence Lifetimes," *Analytical Chemistry* 65, 853-6 (1993).

J.R. Lakowicz, H. Szmacinski, and R.B. Thompson, "Fluorescence Lifetime-Based Sensing: Applications to Clinical Chemistry and Cellular Imaging," in *Proceedings of the SPIE Conference on Ultrasensitive Laboratory Diagnostics, Vol. 1895* (G.E. Cohn, ed.) pp. 2-17 (1993).

R.B. Thompson, "Fiber Optic Ion Sensors Based on Phase Fluorescence Lifetime Measurements," in *Proceedings of the SPIE*

Conference on Advances in Fluorescence Sensing Technology, Vol. 1885 (J.R. Lakowicz and R.B. Thompson, eds.) pp. 290-9 (1993).

J.R. Lakowicz and R.B. Thompson, editors, *Proceedings of the SPIE Conference on Advances in Fluorescence Sensing Technology, Vol. 1885*, 492 pp. (1993).

D.C. Walt (chair), et al., *Applications of Analytical Chemistry to Oceanic Carbon Cycle Studies: A Report to the Commission on Geosciences, Environment, and Resources of the National Research Council*, National Academy Press, 85 pp. (1993).

R.B. Thompson, J.K. Frisoli, and J.R. Lakowicz, "Phase Fluorometry Using a Continuously Modulated Laser Diode," *Analytical Chemistry* 64, 2075-2078 (1992).

R. B. Thompson, H.-H. Zeng, B. P. Maliwal, and C. A. Fierke, "Real time quantitation of Cu(II) by a fluorescence-based biosensing approach," Fifth SPIE Conference on Advances in Fluorescence Sensing Technology, San Jose, CA, January, 2001.

R. B. Thompson, S. W. Suh, C. J. Frederickson, "Ratiometric fluorescence imaging of free zinc in brain," SPIE Conference on Ultrasensitive Clinical Diagnostics, San Jose, CA, January, 2001.

R. B. Thompson, M. Loetz, S. W. Suh, C. J. Frederickson, M. Motamedi, J. Sarvey, Y. Li, C. Hough, W. O. Whetsell, "Quantitative ratiometric imaging of zinc release from organotypic cultures of rat hippocampus," Society for Neuroscience Annual Meeting, New Orleans, November, 2000.

R. B. Thompson, H.-H. Zeng, M. Loetz, C. J. Frederickson, S. W. Suh, "Fluorescence-based metal ion biosensing: Application to imaging zinc release in the hippocampus," 27th Annual Conference of the Federation of Analytical Chemistry and Spectroscopy Societies, Nashville, TN, September, 2000.

R. B. Thompson, "Carbonic anhydrase-based metal ion biosensors: recent progress," Sixth World Congress on Biosensors, San Diego, CA, May, 2000.

R. B. Thompson, H.-H. Zeng, M. Loetz, C. A. Fierke, Issues in enzyme-based metal ion biosensing in complex media," SPIE Conference on In Vitro Diagnostic Instrumentation, San Jose, CA, January, 2000.

R. B. Thompson, H.-H. Zeng, M. Loetz, K. McCall, and C. A. Fierke, "Improved response of a fluorescence-based metal ion

biosensor using engineered carbonic anhydrase variants," SPIE Conference on Advanced Materials and Optical Systems for Chemical and Biological Detection, Boston, September 1999.

R. B. Thompson, B. P. Maliwal, and H.-H. Zeng, "Improved fluorophores for zinc biosensing using carbonic anhydrase," SPIE Conference on New Technologies in Clinical Diagnostics and Drug Discovery, San Jose, CA, February 1999.

R. B. Thompson, B. P. Maliwal, and C. A. Fierke, "Fluorescence-based sensing of transition metal ions by a carbonic anhydrase transducer with a tethered fluorophore," SPIE Conference on Advances in Fluorescence Sensing Technology IV, San Jose, CA, February, 1999.

R. B. Thompson, B. P. Maliwal, and C. A. Fierke, "New carbonic anhydrase-based transducers for metal ion determination by fluorescence anisotropy, " 43rd Annual Meeting of the Biophysical Society, Baltimore, MD, February, 1999.

R. B. Thompson, B. P. Maliwal, and C. A. Fierke, "Biosensing free Cu, Zn, Co, Cd, and Ni at ppb levels in solution by fluorescence anisotropy," 25th Annual Conference of the Federation of Analytical Chemistry and Spectroscopy Societies, Austin, TX, October, 1998.

R. B. Thompson, B. P. Maliwal, V. L. Feliccia, and C. A. Fierke, "Determination of transition metal ions at picomolar levels free in solution with fluorescence polarization."31st Midwestern Regional ACS Meeting, Symposium on Recent Advances in Fluorescence Spectroscopy, Milwaukee, WI, June, 1998.

R. B. Thompson, B. P. Maliwal, V. L. Feliccia, and C.A. Fierke, "Determination of free metal ions in solution at picomolar levels by fluorescence anisotropy," 42nd Annual Meeting of the Biophysical Society, Kansas City, MO, February, 1998

R. B. Thompson, B. P. Maliwal, and V. L. Feliccia, "Determination of metal ions by fluorescence anisotropy exhibits an expanded dynamic range," SPIE Conference on Advances in Optical Biophysics I, San Jose, CA, January 1998

R. B. Thompson, B. P. Maliwal, V. L. Feliccia, and C. A. Fierke, "High sensitivity determination of Zn(II) and Cu(II) *in vitro* by fluorescence polarization," SPIE Conference on Clinical Diagnostic Systems and Technologies, San Jose, CA, January, 1998.

R.B. Thompson, B. P. Maliwal, V.L. Feliccia, C. A. Fierke, C. Frederickson, M. Motamedi, and B. Bell, "Fluorescence-based determination of free Cu(II) and Zn(II)at picomolar levels using

an enzyme transducer," 1997 American Society for Biochemsitry and Molecular Biology Fall Symposium on Copper and Zinc Receptors in Signaling, Trafficking, and Disease, Lake Tahoe, CA, October 1997

R.B. Thompson, B.P. Maliwal, V.L. Feliccia, Z. Ge, M.W. Patchan, C.A. Fierke, and K. McCall, "Determination of metal ions at picomolar levels by fluorescence anisotropy," 1997 Green Chemistry and Engineering Conference, Washington, DC, June, 1997

R.B. Thompson, H.-J. Lin, Z. Ge, K. Johnson, and C.A. Fierke, "Fluorescence lifetime-based determination of cyanide using a carbonic anhydrase-based biosensor, Forty-first Annual Meeting of the Biophysical Society, New Orleans, LA, March, 1997.

R.B. Thompson, H.-J. Lin, Z. Ge, and M.W. Patchan, "Fluorescence lifetime-based biosensing of anions in solutions," SPIE Conference on Advances in Fluorescence Sensing Technology III, February 1997, San Jose, CA.

Z. Ge, R.B. Thompson, and M.W. Patchan, "Fiber Optic Metal Biosensors Based on Fluorescence Energy Transfer of Site-Selective Enzyme Mutants," Pittsburgh Conference on Analytical Chemistry, Chicago, IL, March, 1996.

R.B. Thompson, Z. Ge, M.W. Patchan, and C.A. Fierke, "Fiber optic metal ion biosensors based on fluorescence energy transfer," Fortieth Annual Meeting of the Biophysical Society, Baltimore, MD, February 1996.

R.B. Thompson, Z. Ge, M.W. Patchan, C. Fierke, and C.-C. Huang, "Determination of Multiple Analytes Using a Fiber Optic Biosensor Based on Fluorescence Energy Transfer," SPIE Conference on Ultrasensitive Clinical Diagnostic Systems, BiOS '96, San Jose, CA January 1996.

R.B. Thompson, Z. Ge, M.W. Patchan, L.L. Kiefer, and C.A. Fierke, "Metal Ion Biosensors Based on Fluorescence Energy Transfer," European Science Foundation Conference on Artificial Biosensing Interfaces, Tampere, Finland, June, 1995.

R.B. Thompson, M.W. Patchan, Z. Ge, L.L. Kiefer, C. Fierke, "Fluorescence Energy Transfer-Based Fiber Optic Biosensors," Seventh Conference on Chemical, Biochemical, and Environmental Fiber Optic Sensors, Munich, Germany, June, 1995.

R.B. Thompson, Z. Ge, and M.W. Patchan, "Fiber Optic Metal Ion Biosensors Based on Fluorescence Resonance Energy Transfer," Conference on Lasers and Electro-Optics/Quantum Electronics and Laser Spectroscopy '95, Baltimore, MD, May, 1995.

R.B. Thompson, Z. Ge, and M.W. Patchan, "Use of Site-Selective Mutants in a Fluorescence-Based Fiber Optic Zinc Biosensor," Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, New Orleans, LA, March 1995.

R.B. Thompson, Z. Ge, M.W. Patchan, C.A. Fierke, "Energy Transfer-Based Fiber Optic Metal Ion Biosensor," Second Conference on Advances in Fluorescence Sensing Technology, Photonics West, San Jose, CA, January 1995.

S.J. Froelich-Ammon, M.W. Patchan, N. Osheroff, and R.B. Thompson, "Ellipticine Binding to Topoisomerase II and the Enzyme-DNA Complex," Fifth Conference on DNA Topoisomerases in Therapy, New York, November, 1994.

R.B. Thompson, E.R. Jones, M.W. Patchan, and Z. Ge, "A Fluorescence-Based Fiber Optic Metal Ion Biosensor," Conference on Molecular Recognition, Coolfont, WV, October, 1994.

R.B. Thompson, "Fiber Optic Ion Sensors Based on Phase Fluorescence Lifetime Measurements," SPIE Conference on Fluorescence-Based Sensors, Los Angeles, CA, Jan 1993.